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A survey of fungi in a military aircraft fuel supply system.

## A SURVEY OF FUNGI IN A MILITARY AIRCRAFT FUEL SUPPLY SYSTEM

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### Summary

More than thirty fungi were classified into four groups according to their ability to grow or not in the fuel. Over twenty-three isolates grew.

The microflora of fuels has been investigated rather intensively in the past 10 years. The sudden realization in the late 1950's of the occurrence and importance of microorganisms in aircraft fuels stimulated study of the phenomenon by military and civilian interests. This has resulted in a number of publications. A recent survey of this literature is found in Engel and Swatek (1966).

The few papers describing fungi in fuels (Hedrick et al. 1963, Leathen and Kinsel 1963, Hendey 1964, Bilai et al. 1966) have listed only a few organisms and have not dealt with their relative ability to use fuel for growth. However, it is universally agreed that one organism, *Cladosporium resinae* forma *avellaneum* deVries is the dominant fungus involved in fuel contamination and that this organism is widespread and common in fuels (Hendey 1964).

This paper describes the fungi isolated from a military fuel distribution system at Pease Air Force Base, New Hampshire. The details of the collection of the fuel samples and some results of the survey have been reported by Rogers and Kaplan (1964).

### Materials and Methods

The fungi were collected on Millipore discs at the air base and returned within hours to the laboratory. Fungi were isolated from samples of both the supernatant fuel and the water bottoms as described by Rogers and Kaplan. Pure cultures of the organisms were obtained, assayed for their ability to grow in jet fuel, and rated visually for the amount of growth after 3 weeks incubation at 30°C. Metal-capped culture tubes (16 x 150 mm.) were charged with 5 ml of sterile salt solution\* and overlaid with 2 ml of filter-sterilized fuel. Combustion Ignition Turbine Engine fuel (CITE) was found to be a slightly better substrate for most organisms than JP-4 (a kerosene type fuel) and was selected for the tests. About 800 primary isolations were reduced to less than 300 by comparison and elimination of apparent duplicates. For each strain tested a separate control was run without fuel. For each batch tested a *Cladosporium resinae* control

was also run. Tests of all active cultures were repeated at least once.

### Assay of ability to grow in fuel

The amount of growth was rated visually by comparison with the corresponding control tube. Ratings were assigned on a 5-point scale as follows: 0 = no growth, 1 = trace of growth (equivalent to control tube without fuel), 2 = light growth (visibly greater than control), 3 = moderate growth, 4 = heavy growth (approximately equivalent to the *Cladosporium resinae* control). The medium was inoculated with either a charge of spores or, in non-sporulating strains, a small piece of mycelium from the actively growing edge of an agar culture.

### Results

Although the spectra of organisms which appeared on the various isolation media differed somewhat according to the medium it was not possible to correlate any given medium with a particular fungus. Each medium yielded a variety of strains and each had some strain unique to itself.

Although quantitative data from surveys of this type cannot be equated with similar assays of bacteria where plate counts provide a reasonably accurate estimate of the total population, some indication of the relative abundance of different fungi was obtained. Isolations of *Cladosporium resinae* outnumbered its nearest competitor (*Paecilomyces varioti*) three to one. *Alternaria* was the third most common genus isolated. Species of *Penicillium* and *Aspergillus* were also fairly common. Of the others, isolations ranged from one to a few for each kind. There were a fairly large number of sterile white mycelial forms which were not identifiable as well as a few Phycmycetes and Basidiomycetes.

The results of the fuel utilization studies are presented in Table I with the organisms arranged in four groups according to relative activity. The most active organism was the well known fuel fungus

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\*The salt solution used in the assays contained per liter: 2.6g KH<sub>2</sub>PO<sub>4</sub>, 2.2g K<sub>2</sub>HPO<sub>4</sub>, 3.0g NaNO<sub>3</sub>, 0.25g MgSO<sub>4</sub>·7H<sub>2</sub>O and 0.2g yeast extract. The initial pH was between 6.5 and 7.0. Preliminary experiments showed this solution to be generally better than the Bushnell-Haas solution commonly used in fuel-microbiology studies (Rogers and Kaplan, 1964). The 0.2% yeast extract was sufficient to support the germination of the spores and the production of an extremely small amount of mycelial growth.

*Cladosporium resinae* f. *avellaneum* (Hendey 1964). This was taken as the standard for a visual comparison of the relative growth of the other active cultures.

In addition to providing the greatest amount of growth in the tubes the *Cladosporium* was the only fungus which grew above the fuel-water interface into the fuel layer. Also, this "fuel" growth was darkly pigmented as contrasted to the more hyaline growth in the aqueous phase. Occasionally sporulation also was observed in the fuel phase with *Cladosporium resinae* the only organism for which this was observed.

TABLE I—Fungi Capable of Growing in Fuel

- a. Very Active (visual rating = 4)
  - Cladosporium resinae* f. *avellaneum*
  - Alternaria* "tenuis" group
  - Fusarium* spp.
  - Paecilomyces varioti*
- b. Moderately Active (visual rating = 3)
  - Aspergillus fischeri*
  - A. flavipes*
  - A. fumigatus*
  - A. ustus* group
  - Cladosporium cladosporioides*
  - C. sphaerospermum*
  - Humicola grisea*
  - Ulocladium* sp.
- c. Slightly Active (visual rating = 2)
  - Cephalosporium* sp.
  - Chaetomium globosum*
  - Chaetomium* sp.
  - Curvularia lunata*
  - Epicoccum nigrum*
  - Helminthosporium* sp.
  - Isaria* sp.
  - Monocillium* sp.
  - Penicillium* spp.
- d. Inactive
  - Aspergillus clavatus*
  - A. niger*
  - Aspergillus* spp.
  - Aureobasidium pullulans*
  - Cladosporium herbarum*
  - Curvularia geniculata*
  - Geotrichum candidum*
  - Trichoderma* sp.
  - Several Phycomycetes
  - Several yeasts
  - One Basidiomycete
  - Several sterile white mycelial forms

## Discussion

Of prime importance in the determination of the microbial population in a material such as aircraft fuel is the integrity of the sampling methodology employed. Also very important is prompt isolation and purification of the organisms obtained. The care exercised in this study (see Rogers and Kaplan) assured a high recovery of viable organisms and

avoided the problems inherent in the shipment of samples over long distances or involving extended time lapse between sampling and culturing. In spite of the prompt attention given to the material returned from the field there was some die-off of cultures after transfer to laboratory media.

It is evident that fuel supply systems are capable of harboring an appreciable fungus flora many of which are able to use the fuel for growth. Others, however, are apparently merely inactive dwellers in the fuel-water systems which may even act as a preservative medium for them. The viability of fungi in fuel is variable. Hedrick et al. (1963) found that of six fungi (laboratory cultures not isolated from fuel) tested for survival in a fuel-salts system only *Cladosporium resinae* could be recovered after 126 days. *Alternaria tenuis* was recovered at 105 days, *Aspergillus niger* at 91 days, and the others at 21 days or less.

The fact that an appreciable number of cultures did not survive more than a few days in the laboratory in spite of attempts to keep them alive by the use of different media and incubation conditions suggests that there may be a specialized fungal flora peculiar to the fuel-water systems. The dominance of *Cladosporium resinae* forma *avellaneum* in the fuel system, as indicated by its frequency of isolation in many studies and its very good growth in the fuel, may be due not only to its ability to produce vegetative mycelium but also to its production of conidia *in situ* in liquid culture. No other fungus was observed to sporulate in the assays. This ecological advantage may account for its dominance over other species which, in pure culture, are able to produce significant amounts of mycelium but which are unable to compete with the "kerosene" fungus.

## Conclusion

The fungus flora of a typical military aircraft fuel supply system was found to be quite varied and extensive with many forms capable of using fuel for growth in pure culture. The dominant fungus both in frequency of occurrence and competitive ability in the fuel was *Cladosporium resinae* f. *avellaneum*. Several others (*Alternaria*, *Fusarium*, *Paecilomyces*) are shown in pure culture to produce growth approximately equivalent to the *Cladosporium resinae*, 8 species were able to grow moderately in fuel, and more than 9 species were able to grow weakly in fuel. The failure of the other "very active" fungi to appear in field conditions is believed to be due to the dominance of the strongly competitive *Cladosporium*.

A large number of fungi, incapable of growth in fuel were nevertheless viable when isolated. These included various Fungi Imperfecti, Phycomycetes, several yeasts, one Basidiomycete, and numerous non-fruiting white filamentous forms.

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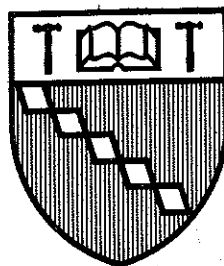
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